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Application of Linkage Disequilibrium Mapping Methods to Detect QTL for Carcass Quality on Chromosome 6 Using a High Density SNP Map in Hanwoo

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ABSTRACT : The purpose of this study was to detect QTL for carcass quality on bovine chromosome (BTA) 6 using a high density SNP map in a Hanwoo population. The data set comprised 45 sires and their 427 Hanwoo steers that were born between spring of 2005 and fall of 2007. The steers that were used for progeny testing in the Hanwoo Improvement Center in Seosan, Korea, were genotyped with the 2,535SNPs on BTA6 that were embedded in the Illumina bovine SNP 50K chip. Four different linkage disequilibrium (LD) mapping models were applied to detect significant SNPs for carcass quality traits; the fixed model with a single marker, the random model with a single marker, the random model with haplotype effects using two adjacent markers, and the random model at hidden state. A total of twelve QTL were detected, for which four, one, three and four SNPs were detected on BTA6 under the respective models ($p<0.001$). Among the detected QTL, four, two, five and one QTL were associated with carcass weight, backfat thickness, *longissimus dorsi* muscle area, and marbling score, respectively ($p<0.001$). Our results suggest that the use of multiple LD mapping approaches may be beneficial in increasing power to detect QTL given a limited sample size and magnitude of QTL effect. (**Key Words :** QTL, Linkage Disequilibrium, Carcass Traits, Hanwoo, SNP)

INTRODUCTION

In the last decade, many advanced technologies have been developed in genomics such as next-generation sequencing and high-throughput genotyping platforms, which provide high-density SNP arrays as a state-of-the-art tool for genetic and genomic analyses in domestic animals (Fan et al., 2010). Due to the developments of high density SNP maps and high-throughput genotyping techniques, marker density is no longer a limiting factor in QTL fine-mapping studies. However, genetic analysis using large amount of SNPs, *e.g.* whole genome association (WGA) study, requires efficient statistical methods (Druet et al., 2008).

Linkage disequilibrium (LD) mapping methods have been practiced for fine QTL mapping studies in livestock species. The efficiency of the LD mapping depends on sample size, SNP map density, magnitude of QTL effect, effective population size, population history, and statistical models (Zhao et al., 2007). To evaluate mapping power and mapping efficiency between LD mapping models , Grapes et al. (2004), Grapes et al. (2006), and Zhao et al. (2007) compared several types of fine LD mapping models with single marker regression, regression based on haplotypes using more than two markers, and identity-by-descent (IBD) mapping. They made a conclusion that the single marker regression analysis had power to detect QTL and mapping precision not worse than the haplotype-based regression or the IBD mapping method. However, Hayes et al. (2007) reported that, when haplotypes with more than four markers were used, the LD mapping method using haplotypes had greater mapping accuracy. Calus et al. (2008) also reported that the IBD approach gave greater accuracy for breeding value estimation than either the single maker regression or the haplotype-based regression method.

Korean consumers prefer a beef of the Korean native cattle, Hanwoo, because the breed has good genetic characteristics in meat palatability and chewiness (Cho and Ko, 1998). In a previous study, we reported three QTL affecting backfat thickness, average daily gain, and final

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weight on BTA6 in a Hanwoo population by applying a linkage mapping method (Lee et al., 2010b). Also, Lee et al. (2008; 2010c) reported that the genotypes of the 12273_165 SNP within the CCDC158 gene are significantly associated with carcass cold weight.

The aim of this study was to further refine the QTL region on BTA6, and to search for candidate genes affecting carcass qualities in Hanwoo by using a high density SNP map and different LD mapping methods.

MATERIALS AND METHODS

Animals, phenotypes and molecular data

The steers (N = 473) with phenotype and molecular data were chosen among the progeny of candidate bulls for progeny testing in the Hanwoo Improvement Center of National Agriculture Cooperative Federation in Seosan, Chungnam province, Korea. The data set comprised 45 sires and their 473 steers that were born between spring of 2005 and fall of 2007. The number of steers for each of the 45 paternal sire families ranged from 6 to 13 with the average eight steers.

The steers were weaned at 5 or 6 months of age, and each group of 10 steers were raised in a pen. The feeding program was divided into early, middle, and late stage, each with six months of interval. In the early and middle stages, the steers were fed with concentrates with the amount of 1.8% of the body weight and ad labium in the late stage. The concentrates were composed of 15%, 13%, and 11% of crude protein, and 71%, 72%, and 73% totally digestible nutrients (TDN), in the respective feeding stages. Roughages with 4.5% crude protein and 37.5 TDN were offered ad labium with other additives such as vitamin and minerals. All steers were slaughtered at an approximately 24 months of age. Traits measured were carcass weight after slaughter (CWT), backfat thickness (BFT), *longissimus dorsi* muscle area (LMA), and marbling score (Marb). The Marb score was numbered as 1 through 9 according to the Korean Beef Marbling Standard (1 = trace, 9 = very abundant). Details about measurement of the traits and management practices were described in Lee et al. (2010a). Table 1 shows the summary statistics for the observed carcass quality traits.

A dense marker map covering BTA6 was constructed with the 2,535 SNPs that were embedded in the Illumina Bovine SNP50K BeadChip (Matukumalli et al., 2009). Details on the SNP genotyping experiments were described in Lee et al. (2010a). Among the 2,535 SNPs, those were removed based on the following criteria: i) monomorphic SNPs or when the minor allele frequency was smaller than 0.05, ii) proportion of individuals with genotype completeness was smaller than 90%, iii) markers with significant departure from H-W equilibrium ($p<0.001$).

QTL analysis

To test the association between a SNP and QTL for carcass quality, the following four models were used.

The fixed model fitting a single marker (Fix_Sig),

$$y_i = \mu + \sum_j \beta_j c_{ij} + \sum_{j=1}^{3} g_{ij\_fixed} + e_i$$

The random model fitting a single marker (Ran_Sig),

$$y_i = \mu + \sum_j \beta_j c_{ij} + sire_i + \sum_{j=1}^{2} g_{ij\_random} + e_i$$

The random model fitting haplotypes using two adjacent markers (Ran_Hap),

$$y_i = \mu + \sum_j \beta_j c_{ij} + sire_i + \sum_{j=1}^{nloc} \sum_{k=1}^{2} haplotype_{ijk} + e_i$$

The random model fitting haplotypes under the hidden states (Ran_HS),

$$y_i = \mu + \sum_j \beta_j c_{ij} + sire_i + \sum_{j=1}^{K} HS_{ij} + e_i \quad ,$$

Where y_i is the phenotypic record of animal i, μ is the average phenotypic performance, $sire_i$ is the random sire effect for animal i, c_{ji} is the value of the j^{th} covariate or fixed effect for the animal i, β_j is an estimate of the j^{th} fixed effect or covariate, g_{ij_fixed} is the fixed effect of SNP genotype values (e.g. -1 for BB, 0 for BA, and 1 for AA) for animal i, g_{ij_random} is the random effect of the SNP alleles (A vs. B) for animal i, $haplotype_{ijk}$ is a random effect for a paternal (k = 1) or maternal (k = 2) haplotype of the two adjacent loci of animal i, HS_{ij} is a random effect for the K

Table 1. Summary statistics of observations on carcass traits for the 427 Hanwoo steers

Trait	Average	Std. Dev.[a]	Minimum	Maximum	CV[b]
Carcass weight (kg)	356	40	158	481	11.1
Backfat thickness (cm)	10.2	0.5	0.3	60	48.8
Longissimus dorsi muscle area (cm^2)	79.1	9.3	22	109	11.8
Marbling score (1-9)	3.4	1.8	1	9	52.2

[a] Standard deviation. [b] Coefficient of variation (%).

defined hidden states for animal *i*, and e_i is a random residual for animal *i*. Construction of haplotype and reconstruction of haplotype hidden states was based on the approach of Druet and Georges (2010). Haplotypes were inferred from the fastPHASE probability model, which is a family rule (Mendelian segregation and linkage) based algorithm. Observed haplotypes were modeled as mosaics of *K* hidden states (or ancestral haplotypes), with *K* held constant throughout the genome (Druet et al., 2008).

Two fixed effects were fitted in the models across all of the fixed and random models; year and season of birth (5 levels) and region where the steers were born (40 levels), and slaughter age was fitted as a covariate. For the fixed model in which a single maker was fitted, phenotypes were preadjusted using SAS GLM procedure of SAS v9.1 (SAS Inst., Inc., Cary, NC), and the residuals of the phenotypes were used in the Fix_Sig model. For the random models, effect of sire was fitted as a random term. The models were tested at each SNP map position on BTA6. The lack of fit (*LOF*) tests was applied to obtain test statistics in the Fix_Sig model (Lee et al., 2010a),

$$LOF = \frac{RSS_{Ho} - RSS_{Ha}}{RSS_{Ho}},$$

where the RSS is the residual sum of squares, and Ho and Ha indicate the models without and with fitting the SNP, respectively (Neter et al., 1990). Threshold values for the *LOF* tests were obtained from the *F* distribution.

For the three random models, likelihood ratio tests (*LRT*) were applied by comparing the maximum likelihoods between the full model with the testing SNP or haplotypes and the reduced model without the QTL effect;

$$LRT = -2(LogLikehood_{reduced\_model} - LogLikehood_{full\_model})$$

The *LRT* test statistics approximately followed chi-squared distributions with 1 degree of freedom (Olsen et al., 2004). For significance threshold, 0.1% comparison-wise p value was applied for QTL detection.

RESULTS AND DISCUSSION

Among the 2,535 SNPs covering BTA6 in the Illumina bovine 50K SNP chip, 1,855 SNPs (73.2%) were available for the association tests. This set of markers covered 122.54 megabase (Mb) of the BTA6 with the 66.04 (±65.04) kb average distance between adjacent markers. A total of 12 QTL were detected, for which four, two, five, and one QTL were detected for CWT, BFT, LMA, and MS, respectively (Table 2). Mapped QTL can be accessed in the Ensembl (Hubbard et al., 2009). Three QTL for CWT were detected in the proximal region of BTA6 (30 to 45 Mb), on which several genes including FAM190A and ABCG2 were

Table 2. Identities, positions of the SNPs associated with carcass traits with statistical significance at the point-wise 0.001 level on BTA6

Trait / SNP Marker[a]	Model[b]	Position(Mb)[c]	-log$_{10}$P[e]	Gene close to Marker
Carcass weight				
BTA-75768-no-rs	Ran_HS	35.25	3.06	FAM190A (35.76Mb-36.10Mb)
BFGL-NGS-114855	Ran_HS	36.09	3.15	FAM190A (35.76Mb-36.10Mb)
BTB-00406718	Ran_Hap	40.84	3.01	SLIT2 (41.19Mb-41.35Mb)
Hapmap27076-BTC-037281		40.89		
ARS-BFGL-NGS-41730	Fix_Sig	122.51	3.29	ABLIM2 (122.44Mb-122.54Mb)
Backfat thickness				
BTB-00244322	Ran_Hap	13.16	3.79	KCC2D (12.89Mb-13.32Mb)
Hapmap40587-BTA-76947		13.23		
Hapmap27519-BTC-047206	Ran_Sig	54.55	3.03	PCDH7 (51.52Mb-51.53Mb)
Longissimus dorsi muscle area				
BTB-00843812	Fix_Sig	55.29	3.62	LOC781512 (57.54Mb-57.79Mb)
BTB-01096127	Fix_Sig	101.41	3.02	SCD5 (101.23Mb-101.41Mb)
ARS-BFGL-NGS-69648	Ran_Hap	110.49	4.63	CLNK (109.94Mb-110.19Mb)
BTB-00280906		110.53		
ARS-BFGL-NGS-32948	Ran_HS	113.58	3.02	BOD1L (113.44Mb-113.46Mb)
ARS-BFGL-NGS-103739	Ran_HS	113.62	3.02	BOD1L (113.44Mb-113.46Mb)
Marbling score				
Hapmap59861-rs29027897	Fix_Sig	42.94	3.12	GPR125 (43.20Mb-43.29 Mb)

[a,c] SNP marker annotations and their positions were based on the bovine reference genome (btau4.0).

[b] Declared LD mapping model type: Fix_Sig = The fixed model fitting a single marker; Ran_Sig = The random model fitting a single marker; Ran_Hap = The random model fitting haplotypes using two adjacent markers; Ran_HS = The random model fitting haplotypes under the hidden states.

[e] Negative logarithm of the comparison-wise p-value of the test statistic against the null hypothesis of no SNP effect at the SNP position.

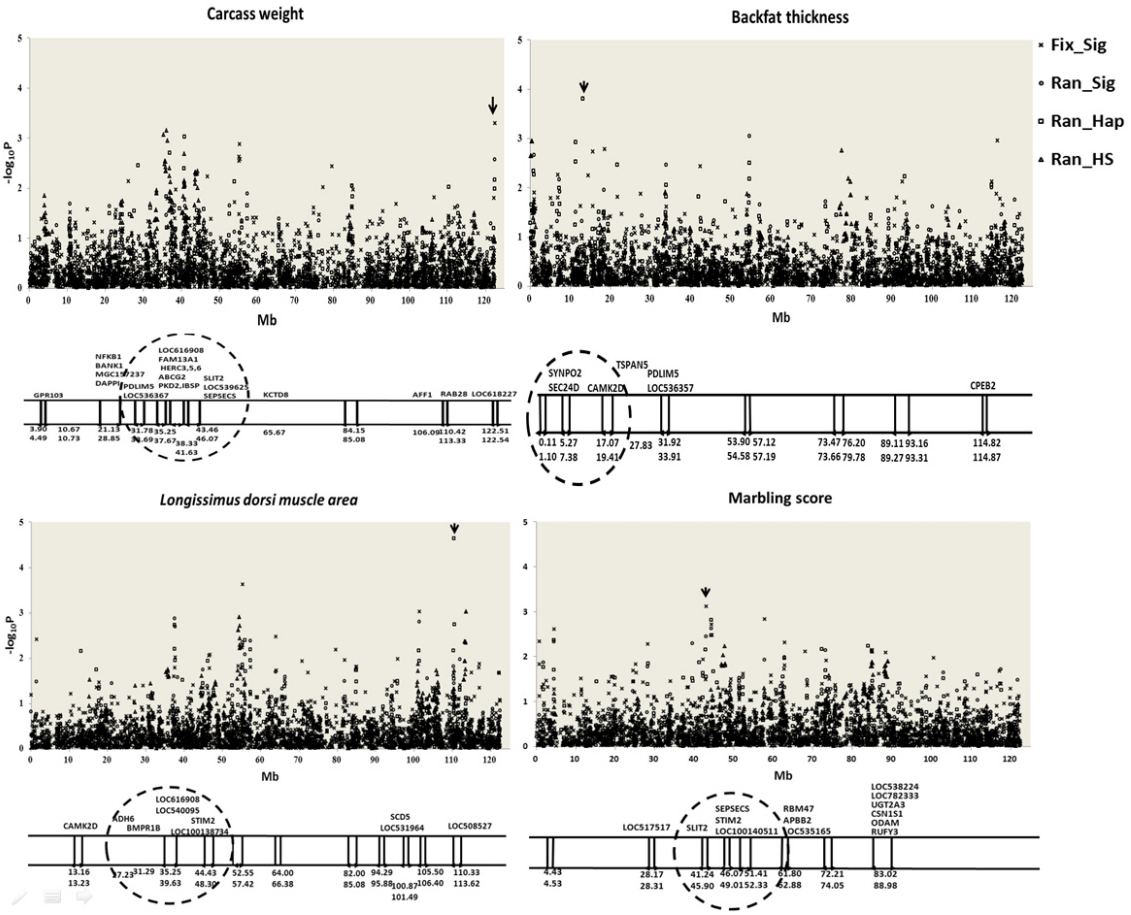

Figure 1. Test statistic profiles and Gene annotations on BTA6. The circle represents the QTL candidate region. The vertical arrows indicated the SNPs, ARS-BFGL-NGS-41730 (Carcass weight), BTB-00244322 and Hapmap40587-BTA-76947 (Backfat thickness), ARS-BFGL-NGS-69648 and BTB-00280906 (*Longissimus dorsi muscle* area), Hapmap59861-rs29027897 (Marbling score) with the most statistical significance on BTA6.

located (Figure 1). The former encodes actin-binding LIM protein family, member 2. A QTL for BFT was detected in the proximal region of the BTA6 (Table 2; Figure 1). In the region, several genes including SYNPO2 and CAMK2D were located, the latter of which encodes calcium/ calmodulin- dependent protein kinase type II delta chain. Four QTL for LMA were detected in the distal region of the test chromosome (100 to 120 Mb), in which one gene, SCD5, that encodes stearyl-CoA desaturase 5, was located. One QTL was detected for Marb in the proximal region of the BTA6 (43Mb), around which several genes such as SLIT2 and STIM2 were located (Figure 1).

The four different LD mapping methods enabled the detection of the four, one, three, and four QTL in the Fix_Sig, Ran_Sig, Ran_Hap, and Ran_HS models, respectively (Table 2 and Figure 1). The QTL was detected only in one of the four mapping models, *i.e.* no QTL was detected in more than two mapping models (Table 2). This may be partly due to the small sample size (N = 473), such that significant QTL in one mapping model can be undetected in another models. Another reason of the inconsistency of QTL detection between the models may be small magnitude of QTL effects. Previously, we scanned BTA6 to find QTL for the carcass quality traits using an interval mapping approach in a Hanwoo population, but only one QTL was detected for BFT at the 5% chromosome-wise level (Lee et al., 2010b). Therefore, our results suggest that the application of multiple LD mapping approaches is beneficial in QTL detection, when QTL effect is of no great magnitude in an experiment with a small sample size. However, care must be taken in determining QTL definition, due to the possibility of false positive QTL.

Some QTL were located in close distances, *e.g.* the two SNPs (BTA-75768-no-rs and BFGL-NGS-114855) for CWT at 35.3 Mb and 36.1 Mb, and the two SNPs (ARS-BFGL-NGS-32948 and ARS-BFGL-NGS-103739) for LMA at 113.6 Mb, respectively (Table 2 and Figure 1). Some of the SNPs may be not causal mutation, *i.e.* the SNPs were so close to the causal SNP as to form high LDs with the causal mutation for the trait.

Threshold values for the statistical analysis were based on the point-wise level, *i.e.* one test basis, and chromosome-wise threshold values were not applied in this study. Because evidences of the QTL for the carcass traits on BTA6 were not strong, *i.e.* the nominal *P* ($-\log_{10}P$) values were greater than 0.00001 (<4.0) (Table 2 and Figure 1), no QTL would be detected when multiple tests were taken into account in this study.

Several QTL studies have been conducted to find any association with carcass quality traits in Hanwoo cattle (Kim et al., 2004; Cheong et al., 2008; Chung et al., 2008). Most reports were based on candidate gene studies, in which genes with physiological functions that were related with the trait of interest were pre-selected and tested. However, there was no report on candidate genes that were located on BTA6 (Cheong et al., 2007; Shin et al., 2007; Cho et al., 2008). Previously, we scanned BTA6 using an interval mapping method in Hanwoo paternal halfsib families (Lee et al., 2010b). Lee et al. (2010b) reported that one QTL for BFT were detected at the 5% chromosome-wise level at 0 cM, which was close to the marker, IL90 (0 cM). Another QTL for LMA was detected at the 10% chromosome-wise level at 67 cM, close to BM4621. In the QTL regions similar to Lee et al. (2010b), we detected QTL for BFT (13.2 Mb) and LMA (55.3 Mb) (Table 2 and Figure 1). In our previous study (Lee et al., 2010a), no significant SNP was found for the carcass quality traits on BTA6 in a Hanwoo population. This may be partly due to the small sample size used in the previous study (N = 289). However, we applied several LD mapping methods with a larger sample (N = 473), and detected twelve QTL for the four carcass quality traits ($p<0.001$).

In conclusion, our results suggest that the use of multiple LD mapping approaches are beneficial in increasing power to detect QTL given a limited sample size and magnitude of QTL effect. The detected QTL need to be verified in other Hanwoo populations for commercial application via marker-assisted selection.

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